REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-19 are in this case. Claims 11-19 were withdrawn under a restriction requirement as drawn to a non-elected invention. Claims 1-10 have been rejected. Claims 6, 7 and 10 have now been canceled. Claims 1-5 and 7-9 have now been amended. New claims 20 and 21 have now been added.

The present invention relates to a method of determining concentration of non-bound metal ions in biological fluids, such as serum samples, which is particularly useful for determining the level of non-transferrin bound iron (NTBI). The method is effected by bringing the sample into contact with a surface coated with a polymer-conjugated form of a metal chelator, which provides multiple binding sites for the binding of metal ions in the sample. Once the metal ions of the sample are bound to the surface through the surface conjugated chelator, the number of remaining available binding sites are quantified by determining the residual capacity of the support-bound metal chelator (e.g., DFO) to bind metal ions (e.g., iron ions). This is effected by using a chelating marker molecule chelating an additional metal ion (i.e., identical to or different from the non-bound metal ion) which can be chelated by the support-bound metal chelator. Thereafter, the amount of chelating marker not chelating the additional metal ion is determined. This amount is indicative of the amount of formerly free metal binding sites in the support, which, in turn, is inversely indicative to the amount of the non-bound metal ion in the sample.

35 U.S.C. § 101 Rejections

The Examiner has rejected claim 10 under 35 U.S.C § 101 because the claimed recitation of a use, without setting forth any steps involved in the process, results in an improper definition of a process, i.e., results in a claim which is not a proper process claim under 35 U.S.C. § 101.

Applicant has now cancelled claim 10, thereby rendering moot Examiner's rejection.

35 U.S.C. § 112, Second Paragraph, Rejections

Claims 1, 3, 5-8 and 10 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiners rejections are respectfully traversed. Claims 6, 7 have now been canceled, rendering moot the Examiner's rejections with respect thereto. Claims 1-5 and 8-9 have now been amended. New claims 20 and 21 have now been added.

With respect to claim 1 step (c), the Examiner points out that the recitation of "a marker conjugated with a moiety that can be captured by the metal chelator" is indefinite because it is not clear whether "a marker" or "a moiety", or both binds to the metal chelator. In addition, the recitation "can be captured" is indefinite because it is unclear whether the method requires actual binding between a metal chelator and "a marker" and/or "a moiety".

With respect to claim 1 step (d), the Examiner points out that the recitations of "the amount" and "marker that has been released" lack antecedent bases because no prior step recites these limitations.

With respect to claim 1, the Examiner points out that the claim is incomplete omitting the step of releasing the marker.

The Examiner further points out with respect to claim 1, that step (d) is indefinite as it is inconsistent with prior steps (b) and (c).

The Examiner further points out with respect to claim 1, that the recitations "binding sites", "the concentration of binding sites", and "the metal ion bound to the marker" lack antecedent bases. In addition, the recitation of "after step b)" is indefinite because it is not clear at what point in the method "after step b)" refers. In addition, the recitation "concentration of binding sites" is indefinite because it is not clear what physical parameters characterize

"concentration of binding sites" or the required steps for determining "concentration of binding sites".

The Examiner further points out that step (e) is inconsistent with prior step (d).

Although Applicant strongly believes that claim 1 as filed accurately represents the present invention, in the interest of expediting prosecution in this case, Applicant has elected to amend claim 1 to include language which better defines the invention of the instant application.

By these amendments, the recitation "after completion of step b)" has been omitted and the term "thereafter" has been added to method claim 1 between each two successive method steps to indicate that the claimed steps are to be executed in the order listed. No new matter has been added.

The recitation "a marker conjugated with a moiety" has been amended to a "chelating marker chelating an additional metal ion". Ample support for this language is found in the specification, wherein an example of a chelating marker is provided in the form of "calcein" a known iron chelator [see page 6 last paragraph, Breuer et al. Breuer et al. (268 Am. J. Physiol. C1354, 1995]. The chelating moiety chelates an additional metal iron which can be identical to the non-bound metal iron (see e.g., page 6 step c), "*e.g., the same metal ion the concentration of which it is desired to determine*") or different therefrom.

Likewise, the recitation "concentration of binding sites left available" has been changed to "said amount of said chelating-marker not chelating said additional metal ion".

Ample support for this is found in the instant application on page 1 reciting in this regard that:

> *"The amount of marker that has been released by capture of the metal ion by the coated surface is determined, and the concentration of the metal ion in the sample is calculated from the*

concentration of binding sites left available for capturing the metal ion bound to the marker."

With respect to claims 5-8, the Examiner points out that the recitations of "marker is a fluorescent marker", "marker is an antibody", "marker is a chromogenic marker" or "marker is a calcein-iron complex" are indefinite since it is not clear how said markers can be "captured by the metal chelator". Claims 6 and 7 have now been cancelled thereby rendering moot the Examiner's rejection of these claims.

Applicant has changed the term "marker" to a "chelating marker" which chelates an additional metal ion, which additional metal ion is chelatable by the metal chelator. An example for such a chelating marker is calcein as indicated by claim 8.

With respect to claim 10, the Examiner points out that since the claim does not set forth any steps involved in the method/process, it is unclear what method Applicant is intending to encompass.

Applicant has cancelled claim 10, thereby rendering moot Examiner's rejection of this claim.

35 U.S.C. § 102 Rejections

The Examiner has rejected claims 1-7 and 9-10 under 35 U.S.C. § 102 as being anticipated by Abuknesha (U.S. Pat. No. 5,723,304). The Examiner's rejections are respectfully traversed.

The Examiner states that Abuknesha teaches a competitive method for determining the concentration of a non-bound metal ion in a sample of serum or other biological fluids in which a polymer-coated surface is conjugated to a metal chelator.

Applicant wishes to point out that the Examiner is mistaken in the assertion that the method described by Abuknesha contemplates the use of

chelator conjugated polymer-coated surface for the detection of metal ions in biological fluids.

In fact, Abuknesha teaches a competitive immunological assay in which a hapten or an antibody (and not a metal chelator as is the case of the present invention) is bound to a solid support for an immunological detection of an analyte of interest using at least two distinct detectable molecules (i.e., detectable species) and calculating a ratio therebetween using any immunoassay configuration.

In brief, the method described by Abuknesha is based on contacting a sample including an analyte of interest with an immobilized molecule (i.e., a part of an immunoaffinity complex such as an antibody, an antigen or a hapten and not a metal-chelator) having an affinity towards the analyte. Thereafter, a first detectable species is added to the reaction mixture allowing to bind to the immobilized analyte, afterwhich a labeled analyte analog (i.e., authentic entity) is added. Such an analyte analog is labeled with a second distinct detectable molecule (i.e., second detectable species). Signal intensities of both detectable molecules are measured and the ratio therebetween is calculated to provide a typical immunoassay curve in which ratio values decrease as the analyte species concentration increases.

Ratio-metric detection of two detectable molecules is the essence of the invention of Abuknesha since, as explained in U.S. Pat. No. 5,723,304, it increases precision of signal measurement and as such increases detection sensitivity.

In sharp contrast to Abuknesha, the present invention relates to a non-competitive method of determining the concentration of a metal ion in a sample, particularly a serum sample, using a single detectable molecule (i.e., marker).

The method of the present invention is effected by contacting the sample with a surface immobilized metal chelator, which provides multiple binding sites for the binding of metal ions in the sample.

Once the metal ions of the sample are bound to the support, the number of remaining available binding sites are quantified by determining the residual capacity of the support-bound metal chelator (e.g., DFO) to bind metal (e.g., iron). This is effected by using a single marker molecule having a metal moiety which can be bound to the support-bound chelator. By way of example, such a marker can be calcein:iron. In this case, calcein is a fluorescent iron binding molecule which fluorescence quenches upon iron binding thereto, and dequenches when iron is removed therefrom. Exposure of the calcein-iron complex to the support-bound chelator leads to transfer of the iron from the calcein-iron complex, due to differences in affinities to the metal ions and thereby to dequenching of the fluorescence of clacein. Hence, the number of metal binding sites in the support is directly proportional to the amount of fluorescence generated from the marker (e.g., calcein-iron). This is in turn, inversely proportional to the amount of metal in the sample.

It is Applicant strong opinion that the use of a single marker (and not two) is advantageous since it may be difficult to differentiate between the signals obtained from the two different detectable molecules such as between two radioactive isotopes, or two different fluorophores, thereby hampering accurate determination of an analyte of interest (see column 11 lines 6-10 of Abuknesha).

Examiner further states that similarly to the instant application, the trapping agent used by the method of Abuknesha is a support-bound metal chelator.

Applicant wishes to point out that the Examiner is mistaken in this case. In fact, in this case, the use of a metal chelator (e.g., deferoxmine) is as a complexing agent to form a detectable entity, which can be immunologically detected by an immobilized antibody (i.e., secondary species).

Thus, in sharp contrast to the method of the present invention, the complexing agent described by Abuknesha et al. does not serve as a trapping agent which is bound to the support for facilitating detection of the analyte

species, rather it is complexed with the analyte species to be detected immunologically by an antibody trapping agent which is bound to the support. To this end, see column 13, lines 6 – 9 of the instant specification, stating that:

> For example, where an analyte species is a metal ion the agent may be a complexing agent capable of interacting with the metal ion to form an entity (e.g., a metal ion complex) for detection. (Emphasis added)

Thus, Applicant is of the strong opinion that Abuknesha does not describe, nor does he suggest a method of determining the concentration of a non-bound metal ion using a single reporter, as provided by the method of the instant invention, and as such cannot, and does not anticipate the method of the present invention.

35 U.S.C. § 103(a) Rejections

The Examiner has rejected claim 8 under 35 U.S.C. § 103(a) as being unpatentable over Abuknesha (U.S. Pat. No. 5,723,304) in view of Breuer et al. (268 Am. J. Physiol. C1354, 1995).

The Examiner states that Breuer et al. teach the use of a calcein-iron marker for measuring intracellular iron concentrations. Therefore, it would have been obvious for a person of ordinary skill in the art to perform the method of determining the concentration of a non-bound metal ion, as taught by Abuknesha, with the clacein-iron marker, as taught by Breuer et al.

As is extensively argued hercinabove with respect to the 102 rejections, Abuknesha discloses a method for determining analyte species using two detectable labels, which is clearly distinct from the present approach both in the method steps employed and in the approach taken for iron quantification.

With respect to Breuer et al., Applicant would like to further point out that dequenching fluorescence of calcein:iron complexes by chelators (i.e., DFO) is described extensively in the art. However, in all these cases, calcein is

the primary iron-binding agent (always within cells) and its dequenching is an indication of how much iron was bound to the calcein.

However, since calcein is a relatively weak iron chelator (as compared to DFO) it cannot be used for measuring NTBI in sera, because it is unable to compete with other ligands in the system (e.g., oxalate, nitrilotriacetate or even endogenous albumin, phosphate or citrate). This requires a strong chelator such as DFO. Calcein is only useful as a primary iron detector of intracellular iron where the competing ligands are not as efficient as those in the serum and where the iron forms are different than those present in the serum [i.e., divalent Fe (II) rather than trivalent Fe (III)].

Thus, the system of calcein:iron can be used for measuring a portion of labile intracellular iron, but is not applicable for NTBI quantification where higher affinity ligands are required.

Therefore, the role of calcein:iron complexes in the instant invention is as a marker, to quantify support-polymer conjugated DFO (chelator) and not to directly quantify the amount of available iron in serum samples.

Thus, it is Applicant's strong opinion that in view of the profound differences between the present invention and the method taught by Abuknesha, and due to the fact that Breuer et al. merely teaches determination of intracellular labile iron, the combined teachings of Abuknesha and Breuer et al. would not guide or motivate one of ordinary skill in the art to make the present invention and thus these prior art references do not alone or in combination render obvious the present invention

In view of the above amendments and remarks it is respectfully submitted that claims 1-5, 7-9 and 20-21 are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,

Sol Sheinbein
Registration No. 25,457

Date: March 20, 2005.

Encl.:
Two months extension fees.